

PATENT  
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Docket 091/005

CLAIM AMENDMENTS

1. *(Previously presented)* A method for producing a population of genetically altered human embryonic stem (hES) cells, comprising:
  - a) obtaining a population of hES cells essentially free of feeder cells; and
  - b) transfecting the cells with a polynucleotide while being cultured on an extracellular matrix in a medium conditioned by fibroblast feeder cells, wherein the polynucleotide comprises a protein encoding region operably linked to a promoter that promotes transcription of the encoding region while the cells are undifferentiated,  
thereby producing genetically altered hES cells that express the protein while undifferentiated.
2. *(Original)* The method of claim 1, further comprising preferentially selecting cells that have been genetically altered with the polynucleotide.
3. *(Previously presented)* The method of claim 1, wherein the human embryonic stem cells are maintained in an environment comprising extracellular matrix components and a conditioned medium produced by collecting medium from a culture of feeder cells.

4 & 5. CANCELLED

6. *(Previously presented)* The method of claim 1, wherein the polynucleotide is selected from an adenoviral vector, a retroviral vector, and a DNA plasmid complexed with positively charged lipid.
7. CANCELLED
8. *(Currently amended)* A cell population comprising undifferentiated human embryonic stem (hES) cells essentially free of feeder cells, cultured on an extracellular matrix in a medium conditioned by fibroblast feeder cells,  
wherein the population comprises cells expressing a protein from a heterologous polynucleotide in which an encoding region for the expressed protein is operably linked to a promoter that promotes transcription of the encoding region while the hES cells are undifferentiated.

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9. *(Currently amended)* A cell population comprising undifferentiated hES cells essentially free of feeder cells, cultured on an extracellular matrix in a medium conditioned by fibroblast feeder cells, wherein the population comprises cells stably transfected so as to express a protein from a heterologous polynucleotide in which an encoding region for the expressed protein is operably linked to a promoter that promotes transcription of the encoding region while the hES cells are undifferentiated.

10 to 12. **CANCELLED**

13. *(Previously presented)* The cell population of claim 8, in which at least 90% of the undifferentiated hES cells have been genetically altered.

14. **CANCELLED**

15. *(Previously presented)* The cell population of claim 9, in which at least 90% of the undifferentiated hES cells have been stably transfected.

16. *(Previously presented)* A method for producing genetically altered differentiated cells, comprising differentiating the cells of claim 9.

17. *(Previously presented)* A method for producing genetically altered differentiated cells, comprising:

- a) obtaining a population of hES cells essentially free of feeder cells and maintained on an extracellular matrix in a medium conditioned by fibroblast feeder cells; and
- b) transfecting at least some of the cells in the composition with a polynucleotide, thereby producing genetically altered cells; and
- c) causing the genetically altered cells to differentiate into a population of neural cells or hepatocytes.

18. *(Previously presented)* The method of claim 16, whereby the genetically altered cells are differentiated into neural cells.

19. *(Previously presented)* The method of claim 16, whereby the genetically altered cells are differentiated into hepatocytes.

20. *(Previously presented)* The method of claim 17, whereby the differentiated cell population is over 50% neural cells.

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21. *(Previously presented)* The method of claim 17, whereby the differentiated cell population is over 50% hepatocytes.
22. *(Previously presented)* The method of claim 1, wherein the polynucleotide encodes a drug resistance gene.
23. *(Previously presented)* The method of claim 2, wherein the selecting comprises culturing the cells in the presence of a drug to which genetically altered cells in the population are resistant.
24. *(Previously presented)* The method of claim 1, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
25. *(Previously presented)* The cell population of claim 8, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
26. *(Previously presented)* The cell population of claim 9, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
27. *(Previously presented)* The cell population of claim 8, which consists of human cells.
28. *(Previously presented)* The cell population of claim 9, which consists of human cells.
29. *(Previously presented)* The cell population of claim 8, wherein the protein is a factor that supports growth of the hES cells.
30. *(Previously presented)* The cell population of claim 29, wherein the protein is a fibroblast growth factor.
31. *(Previously presented)* The cell population of claim 8, wherein the protein is a detectable label.
32. *(Previously presented)* The cell population of claim 31, wherein the label is a fluorescent label.
33. *(Previously presented)* The cell population of claim 32, wherein the label is selected from luciferase and green fluorescent protein (GFP).
34. *(Previously presented)* The cell population of claim 31, wherein the label is a cell surface protein detectable by antibody staining.

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35. *(Previously presented)* The cell population of claim 31, wherein the label is an enzyme.
36. *(Previously presented)* The cell population of claim 35, wherein the label is selected from alkaline phosphatase,  $\beta$ -galactosidase, and neophosphotransferase.